PATENT
Docket No.: 2026-4199US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Charles R. VINSON and Dmitry KRYLOV
Serial No.	:	Divisional of U.S. Serial No. 09/299,495
Filed	:	January 29, 2002
For	:	**EXTENSION OF A PROTEIN-PROTEIN INTERACTION SURFACE TO INACTIVATE THE FUNCTION OF A CELLULAR PROTEIN**
Group Art Unit	:	To Be Assigned
Examiner	:	To Be Assigned

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Preliminary to the examination of the above-identified divisional patent application, entry and consideration of this Preliminary Amendment are respectfully requested.

IN THE CLAIMS:

Please amend claim 28 as follows:

28. (Amended) A method of creating a transgenic plant or non-human animal containing a gene encoding an expressible dominant negative protein to a naturally occurring cellular protein, comprising:

(a) introducing into a cell of the of the plant or non-human animal an isolated DNA molecule encoding an acidically modified nucleic acid binding

protein containing an N-terminal extension of acidic amino acid residues, thereby forming an acidic N-terminal extension of a protein-protein interaction surface or of a dimerization or multimerization surface of said acidically modified nucleic acid binding protein, said acidic N-terminal extension allowing said acidically modified nucleic acid binding protein to dimerize or multimerize with a normal cellular protein, under conditions allowing for the expression of said acidically modified nucleic acid binding protein in cells of the plant or non-human animal; and

(b) expressing the acidically modified nucleic acid binding protein in the cells of the plant or non-human animal, thereby allowing multimeric or dimeric complexation between said expressed acidically modified nucleic acid binding protein and a cognate naturally occurring cellular protein.

Please add the following new claims 29-56:

29. (New) The method according to claim 28, wherein the isolated DNA molecule is introduced into the cells in a vector.

30. (New) The method according to claim 29, wherein the vector is a prokaryotic, eukaryotic or viral vector.

31. (New) The method according to claim 29, wherein the vector comprises a cell- or tissue-specific promoter, being inducible or noninducible, a transcription initiation site, a transcription termination site, an origin of replication, and a polyadenylation site, for expression in host cells.

32. (New) The method according to claim 30, wherein the vector

is selected from the group consisting of Simian Virus 40 (SV40) vector, adenovirus vector, vaccinia virus vector and adeno-associated virus vector.

33. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein is an acidically modified bZIP protein.

34. (New) The method according to claim 33, wherein the encoded acidically modified bZIP protein is selected from the group consisting of Fos, Jun, GCN4 (general control of nitrogen and purine metabolism factor-4), VBP (vitellogenin gene binding protein), GBF-1 (G-box factor-1), opaque, DBP (D-box binding protein), CHOP-10 (C/EBP homologous protein-10), CREB (CRE binding protein), C/EBP (CCAAT/enhancer binding protein), PAR (proline- and acidic amino acid-rich protein), and ATF2 (activating transcription factor-2).

35. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein is an acidically modified basic helix-loop-helix (bHLH) protein.

36. (New) The method according to claim 35, wherein the encoded acidically modified bHLH protein is selected from the group consisting of c-myc, n-myc, i-myc, max, mad, ID, MyoD1 (myogenic differentiation factor-1), E12 (immunoglobulin enhancer binding protein-12), AP-4 (activating enhancer binding protein-4), TFE3 (transcription factor E3), USF (upstream stimulatory factor), and FIP (Fos interacting protein).

37. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein has an amino acid sequence as shown in any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:57, or SEQ ID NO:64.

38. (New) A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant acidic dominant negative polynucleotide sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage, wherein the expression product of said acidic dominant negative sequence stably dimerizes or multimerizes with a normal cellular protein.

39. (New) The mammal according to claim 38, wherein transcription of said acidic dominant negative polynucleotide sequence is controlled by a tissue-specific promoter sequence.

40. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42,

SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:57 and SEQ ID NO:64.

41. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence is selected from the group consisting of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:32.

42. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence encodes a 3heptadF- (SEQ ID NO:37) C/EBP sequence (SEQ ID NO:58).

43. (New) The mammal according to claim 39, wherein the promoter sequence is an adipose fatty acid binding protein promoter sequence.

44. (New) The mammal according to claim 43, wherein the adipose fatty acid binding protein promoter sequence is aP2.

45. (New) The mammal according to claim 43, wherein the adipose fatty acid binding protein promoter sequence controls gene expression in adipose tissue.

46. (New) The mammal according to claim 39, wherein the promoter sequence is a whey activating protein promoter sequence.

47. (New) The mammal according to claim 46, wherein the whey activating promoter sequence controls gene expression in mammary or breast

tissue.

48. (New) The mammal according to claim 38, said mammal being selected from the group consisting of rodents, sheep, pigs and primates.

49. (New) The mammal according to claim 38, said mammal being a rodent.

50. (New) The mammal according to claim 49, said rodent being a mouse or a rat.

51. (New) A transgenic non-human mammal whose germ cells and adipose tissue cells contain an expression system comprising:

(a) a first DNA sequence coding on expression for an acidic dominant negative b-Zip or bHLH protein; said acidic dominant negative protein having a biological activity of a dominant negative to a cellular protein; and

(b) a promoter functional in said adipose tissue cells, whereby said mammal, under conditions conducive to expression, expresses said acidic dominant negative protein in adipose tissue of said mammal.

52. (New) The transgenic non-human mammal according to claim 51, wherein the acidic dominant negative b-Zip or bHLH protein comprises an N-terminal acidic amino acid sequence extension selected from the group consisting of SEQ ID NOS:35-42.

53. (New) The transgenic non-human mammal according to claim

51, wherein is acidic dominant negative b-Zip protein is 3heptadF-C/EBP.

54. (New) A method of producing a transgenic non-human mammal capable of expressing a protein which has a biological activity of an acidic dominant negative to a cellular protein, said method comprising chromosomally incorporating a polynucleotide sequence encoding the protein into the genome of a non-human mammal so that said genome comprises an expression system comprising:

(a) a first DNA sequence coding on expression for an acidic dominant negative b-Zip or bHLH protein; said acidic dominant negative protein having a biological activity of a dominant negative to a cellular protein; and

(b) a promoter functional in adipose tissue cells, whereby said mammal, under conditions conducive to expression, expresses said acidic dominant negative protein in adipose tissue of said mammal.

55. (New) The method according to claim 54, wherein the acidic dominant negative b-Zip or bHLH protein comprises an N-terminal acidic amino acid sequence extension selected from the group consisting of SEQ ID NOS:35-42.

56. (New) A transgenic non-human mammal which is a founder mammal prepared by the method of claim 54, or progeny of said founder mammal, wherein said transgenic mammal, under conditions conductive to expression, expresses said protein in adipose tissue of said mammal so as to affect the normal production of fat tissue.

REMARKS

In this Preliminary Amendment, claim 28 has been amended and new claims 29-56 have been added. The amended and new claims are supported by the application as filed, and no new matter has been introduced into the application by virtue of the amended and new claims. Specifically, the claims find support in the original claims and in the disclosure of the specification in Examples 13 and 14. Accordingly, the presently pending claims are 28-56. The amendments made to claim 28 are presented in the attached page entitled "Version with Markings to Show Changes Made".

AUTHORIZATION

Should fee(s) additional to those paid be deemed necessary for the filing of this application, including the Preliminary Amendment, the Commissioner is hereby authorized to charge any fee(s) which may be properly assessable in this application to Deposit Account No. 13-4500, Order No. 2026-4199US3.

Respectfully submitted,

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Date: January 29, 2002

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Version with Markings to Show Changes Made

Claim 28 has been amended as follows:

28. (Amended) A method of [controlling cell growth by inhibiting the function of a naturally occurring cellular protein] creating a transgenic plant or non-human animal containing a gene encoding an expressible dominant negative protein to a naturally occurring cellular protein, comprising:

(a) introducing into a cell of the of the plant or non-human animal an isolated DNA molecule encoding an acidically modified nucleic acid binding protein containing an N-terminal extension of acidic amino acid residues, thereby forming an acidic N-terminal extension of a protein-protein interaction surface or of a dimerization or multimerization surface of said acidically modified nucleic acid binding protein, said acidic N-terminal extension allowing said acidically modified nucleic acid binding protein to dimerize or multimerize with a normal cellular protein, [the construct according to claim 18] under conditions allowing for the expression of said acidically [extended] modified nucleic acid binding protein in cells of the plant or non-human animal; and

(b) expressing the acidically modified nucleic acid binding protein in the cells of the plant or non-human animal, thereby allowing [inhibiting the binding of a cognate naturally occurring cellular protein to its target nucleic acid sequence by] multimeric or dimeric complexation between said expressed acidically [extended] modified nucleic acid binding protein and [said] a cognate naturally occurring cellular protein.

Currently Pending Claims

Currently pending claims 28-56 read as follows:

28. (Amended) A method of creating a transgenic plant or non-human animal containing a gene encoding an expressible dominant negative protein to a naturally occurring cellular protein, comprising:

(a) introducing into a cell of the of the plant or non-human animal an isolated DNA molecule encoding an acidically modified nucleic acid binding protein containing an N-terminal extension of acidic amino acid residues, thereby forming an acidic N-terminal extension of a protein-protein interaction surface or of a dimerization or multimerization surface of said acidically modified nucleic acid binding protein, said acidic N-terminal extension allowing said acidically modified nucleic acid binding protein to dimerize or multimerize with a normal cellular protein, under conditions allowing for the expression of said acidically modified nucleic acid binding protein in cells of the plant or non-human animal; and

(b) expressing the acidically modified nucleic acid binding protein in the cells of the plant or non-human animal, thereby allowing multimeric or dimeric complexation between said expressed acidically modified nucleic acid binding protein and a cognate naturally occurring cellular protein.

29. (New) The method according to claim 28, wherein the isolated DNA molecule is introduced into the cells in a vector.

30. (New) The method according to claim 29, wherein the vector is a prokaryotic, eukaryotic or viral vector.

31. (New) The method according to claim 29, wherein the vector comprises a cell- or tissue-specific promoter, being inducible or noninducible, a transcription initiation site, a transcription termination site, an origin of replication, and a polyadenylation site, for expression in host cells.

32. (New) The method according to claim 30, wherein the vector is selected from the group consisting of Simian Virus 40 (SV40) vector, adenovirus vector, vaccinia virus vector and adeno-associated virus vector.

33. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein is an acidically modified bZIP protein.

34. (New) The method according to claim 33, wherein the encoded acidically modified bZIP protein is selected from the group consisting of Fos, Jun, GCN4 (general control of nitrogen and purine metabolism factor-4), VBP (vitellogenin gene binding protein), GBF-1 (G-box factor-1), opaque, DBP (D-box binding protein), CHOP-10 (C/EBP homologous protein-10), CREB (CRE binding protein), C/EBP (CCAAT/enhancer binding protein), PAR (proline- and acidic amino acid-rich protein), and ATF2 (activating transcription factor-2).

35. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein is an acidically modified basic helix-loop-helix (bHLH) protein.

36. (New) The method according to claim 35, wherein the

encoded acidically modified bHLH protein is selected from the group consisting of c-myc, n-myc, i-myc, max, mad, ID, MyoD1 (myogenic differentiation factor-1), E12 (immunoglobulin enhancer binding protein-12), AP-4 (activating enhancer binding protein-4), TFE3 (transcription factor E3), USF (upstream stimulatory factor), and FIP (Fos interacting protein).

37. (New) The method according to claim 28, wherein the encoded acidically modified nucleic acid binding protein has an amino acid sequence as shown in any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:57, or SEQ ID NO:64.

38. (New) A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant acidic dominant negative polynucleotide sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage, wherein the expression product of said acidic dominant negative sequence stably dimerizes or multimerizes with a normal cellular protein.

39. (New) The mammal according to claim 38, wherein transcription of said acidic dominant negative polynucleotide sequence is controlled by a tissue-specific promoter sequence.

40. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:57 and SEQ ID NO:64.

41. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence is selected from the group consisting of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:32.

42. (New) The mammal according to claim 38, wherein the acidic dominant negative polynucleotide sequence encodes a 3heptadF- (SEQ ID NO:37) C/EBP sequence (SEQ ID NO:58).

43. (New) The mammal according to claim 39, wherein the promoter sequence is an adipose fatty acid binding protein promoter sequence.

44. (New) The mammal according to claim 43, wherein the adipose fatty acid binding protein promoter sequence is aP2.

45. (New) The mammal according to claim 43, wherein the adipose fatty acid binding protein promoter sequence controls gene expression in

adipose tissue.

46. (New) The mammal according to claim 39, wherein the promoter sequence is a whey activating protein promoter sequence.

47. (New) The mammal according to claim 46, wherein the whey activating promoter sequence controls gene expression in mammary or breast tissue.

48. (New) The mammal according to claim 38, said mammal being selected from the group consisting of rodents, sheep, pigs and primates.

49. (New) The mammal according to claim 38, said mammal being a rodent.

50. (New) The mammal according to claim 49, said rodent being a mouse or a rat.

51. (New) A transgenic non-human mammal whose germ cells and adipose tissue cells contain an expression system comprising:

(a) a first DNA sequence coding on expression for an acidic dominant negative b-Zip or bHLH protein; said acidic dominant negative protein having a biological activity of a dominant negative to a cellular protein; and

(b) a promoter functional in said adipose tissue cells, whereby said mammal, under conditions conducive to expression, expresses said acidic dominant negative protein in adipose tissue of said mammal.

52. (New) The transgenic non-human mammal according to claim 51, wherein the acidic dominant negative b-Zip or bHLH protein comprises an N-terminal acidic amino acid sequence extension selected from the group consisting of SEQ ID NOS:35-42.

53. (New) The transgenic non-human mammal according to claim 51, wherein is acidic dominant negative b-Zip protein is 3heptadF-C/EBP.

54. (New) A method of producing a transgenic non-human mammal capable of expressing a protein which has a biological activity of an acidic dominant negative to a cellular protein, said method comprising chromosomally incorporating a polynucleotide sequence encoding the protein into the genome of a non-human mammal so that said genome comprises an expression system comprising:

(a) a first DNA sequence coding on expression for an acidic dominant negative b-Zip or bHLH protein; said acidic dominant negative protein having a biological activity of a dominant negative to a cellular protein; and

(b) a promoter functional in adipose tissue cells, whereby said mammal, under conditions conducive to expression, expresses said acidic dominant negative protein in adipose tissue of said mammal.

55. (New) The method according to claim 54, wherein the acidic dominant negative b-Zip or bHLH protein comprises an N-terminal acidic amino acid sequence extension selected from the group consisting of SEQ ID NOS:35-42.

56. (New) A transgenic non-human mammal which is a founder mammal prepared by the method of claim 54, or progeny of said founder mammal, wherein said transgenic mammal, under conditions conductive to expression, expresses said protein in adipose tissue of said mammal so as to affect the normal production of fat tissue.